Swimming microorganisms acting as nanorobots versus artificial nanorobotic agents: A perspective view from an historical retrospective on the future of medical nanorobotics in the largest known three-dimensional biomicrofluidic networks

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The vascular system in each human can be described as a 3D biomicrofluidic network providing a pathway close to approximately 100 000 km in length. Such network can be exploited to target any parts inside the human body with further accessibility through physiological spaces such as the interstitial microenvironments. This fact has triggered research initiatives towards the development of new medical tools in the form of microscopic robotic agents designed for surgical, therapeutic, imaging, or diagnostic applications. To push the technology further towards medical applications, nanotechnology including nanomedicine has been integrated with principles of robotics. This new field of research is known as medical nanorobotics. It has been particularly creative in recent years to make what was and often still considered science-fiction to offer concrete implementations with the potential to enhance significantly many actual medical practices. In such a global effort, two main strategic trends have emerged where artificial and synthetic implementations presently compete with swimming microorganisms being harnessed to act as medical nanorobotic agents. Recognizing the potentials of each approach, efforts to combine both towards the implementation of hybrid nanorobotic agents where functionalities are implemented using both artificial/synthetic and microorganism-based entities have also been initiated. Here, through the main eras of progressive developments in this field, the evolutionary path being described from some of the main historical achievements to recent technological innovations is extrapolated in an attempt to provide a perspective view on the future of medical nanorobotics capable of targeting any parts of the human body accessible through the vascular network. © *2016 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4945734]

INTRODUCTION

Unlike medical microrobots which are entities referred as such mainly because of their overall dimensions in the micrometer (μm) range, medical nanorobots (nanobots) are usually defined as devices or agents (nanorobotic agents) aimed at medical applications with overall dimensions in the nanometer (nm) range (nanoscale nanorobotic agents) or agents ranging in sizes from a fraction of a micrometer to a few micrometers (typically up to a maximum of a few tens of micrometers) that exploit physical phenomena occurring only at the nanoscale (nanotechnology) by embedding the associated nano-components for the implementation of particular robotic functionalities (microscale nanorobotic agents). Medical nanorobotics is a relatively new field of research that most recently have shown a significant increase in the number of demonstrations and proof-of-concepts. Such recent demonstrations may suggest a not-to-far

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adoption of these new technologies in medical practices and particularly among other possible interventions, for targeting physiological areas accessible only through the vast human vascular network.

The history and the future development of medical nanorobotics are divided here for the first time as specific eras, as depicted in Fig. 1. It begins with the preamble era where few events would later inspire and influence the development of modern medical nanorobotics. The preamble era where no activities in the field of nanorobotics took place was followed in 1959 by the conceptual era. It is during the conceptual era that the first thoughts towards the implementation of medical nanorobotics defining the theoretical foundations were initiated. Such era would not only inspire and show the possibility of implementing various forms of more futuristic nanorobotic agents but would also define laws of physics that would prove to be an essential part of the accelerated development that would soon follow. In parallel to the conceptual era

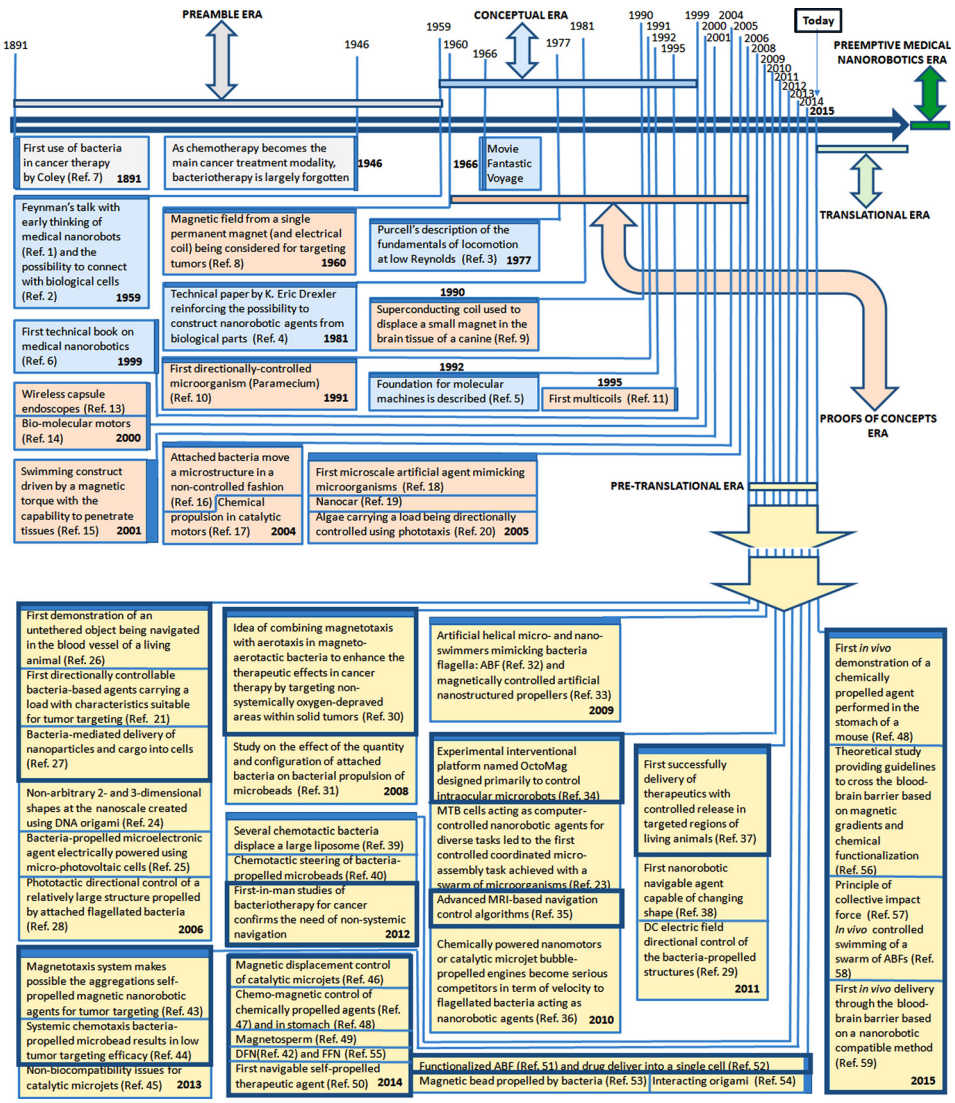


FIG. 1. The history of medical nanorobotics being represented through several eras—As depicted, the pre-translational era shows a significant increase in the number of demonstrations and proofs of concepts that are directly aimed towards medical interventions (identified in boxes having a thicker frame). This fact suggests that medical nanorobotics may soon begin to influence specific medical practices. Notice that further proofs of concepts were done during the pre-translational era, and further proofs of concepts will likely be demonstrated beyond the pre-translational era. These overlaps show that each era reflects more the level of maturity of the research efforts, while other activities from previous eras continue to be pursued.

was the proofs-of-concepts era where more practical demonstrations were taking place. This proofs-of-concepts era was followed by the pre-translational era where most research activities were aimed specifically to one or more particular medical interventions. Indeed, it is during this pre-translational era that the field of medical nanorobotics began to show an increasing number of experimental demonstrations. These demonstrations would not only amplify the level of enthusiasm towards more research and development activities in the field but would also suggest that the time when the first medical applications could benefit from such technologies would appear to be not as far as previously anticipated.

Presently, we are at the end of this pre-translational era and are initiating the translational era where the perspective view of the future medical nanorobotics being described in more details later, really starts. During such translational era, it is anticipated based on the level of technological maturity achieved through the previous eras, that medical nanorobotics will take an increasingly important and strategic role in many future medical practices. Ultimately, research and development will continue to go beyond medical interventions towards a more preemptive role that after a long period of efforts will lead to the preemptive medical nanorobotics era.

In order to provide a feel about the rate at which the field had progressed in order to attempt to predict its future progression, this paper highlights some of the main historical achievements that contributed to lead nanorobotics towards future medical applications and in particular, the ones that need targeting through the vascular network. Targeting tumoral regions in cancer therapy in one particular example of medical intervention that could be enhanced significantly with nanorobotic agents navigated in the vascular network. Therefore, to remain focus on the achievements leading nanorobotics towards the first medical interventions, we are interested here more specifically on the ones that involve particular fields of robotics including but limited to actuation and navigation. In other words, we are more interested here in nanorobotic agents that can be navigated from one location to a specific target typically using the shortest physiological route instead of molecular constructs and related fundamental fields that could contribute to the synthesis of nanorobotic agents but without navigation capability, i.e., intended for systemic circulation in the vascular network which is known to be a major drawback in drug delivery.

THE CONCEPTUAL AND PREAMBLE ERAS

Early thinking in medical nanorobotics includes the 1959 talk "There's Plenty of Room at the Bottom," by Nobel physicist Feynman that proposed employing machine tools to make smaller machine tools, these to be used in turn to make still smaller machine tools, and so on all the way down to the atomic level.[1] Also of interest is the fact that Feynman added remarks on potential medical applications. But what is also interesting is that soon after this historical lecture, Feynman urged to consider the possibility to connect with biological cells.[2]

Two decades later in 1981, Purcell published in 1977 an important paper[3] describing the fundamentals of locomotion at low Reynolds number. This paper would later inspire future actuation design of artificial micro- and nanorobotic agents mimicking microorganisms. Then, the vision of Feynman was reinforced in a technical paper[4] by Drexler suggesting the possibility to construct such nanorobotic agents from biological parts. Another decade passed before the foundations for molecular machine systems were described[5] in 1992 prior to the first technical book[6] on medical nanorobotics in 1999. These conceptual ideas that aimed at describing more futuristic agents than the ones that were pursued in parallel during the proofs-of-concepts era (described in "The Proofs-of-Concepts Era" section), followed a period being referred to here as the preamble era. It is during the preamble era that some events would influence later to some degrees, the development of medical nanorobotics. One of these events was the use of bacteria in cancer therapy. The use of bacteria in cancer therapy was based on initial observations reported by Coley in 1891.[7] Although not a single robotic task such as the displacement control of the bacteria was done, these first observations suggested the possibility of using microorganisms to treat cancer. This led the way to future implementations of bacteria-based

nanorobotic agents for cancer treatments. But starting in 1946, chemotherapy gradually became the principal therapeutic strategy in cancer, and bacterial therapies began to be largely forgotten until it began to attract attention again in recent years.

THE PROOFS-OF-CONCEPTS ERA

It is in 1960 that for the first time, magnetic field was considered for targeting.[8] The use of an external magnet (permanent magnet or electromagnetic coil) was very limited by its inefficacy to target in deep tissues due to the rapid decay of the magnetic field while not providing navigation capability. Nonetheless, it was a precursor to the more advanced nanorobotic magnetic navigation platforms that would follow. It took another 30 years before a small permanent magnet was displaced *in vivo* in the brain tissue of a canine by a single movable superconductive coil.[9] The superconducting coil being considered for the first time in 1990 provided a much higher gradient deeper in tissues but had a scaling issue. Scaling for clinical uses was an issue, since superconducting magnets were too bulky to be practically moved around a human adult. Furthermore, despite the higher magnetic field being produced, this platform was still not appropriated to navigate medical nanorobotic agents. Indeed, navigating such agents in the vascular network often requires fast directional changes that cannot be done when relying on mechanical-based displacements of one or more heavy magnetic sources. Furthermore, the field strength although higher than using a non-superconducting magnet was still insufficient to bring superparamagnetic iron-oxide nanoparticles (SPIONs) at saturation magnetization in order to maximize the effect of the gradient fields within a workspace required to conduct whole-body operations.

This is one example among others where nanoscale components play a crucial role in medical nanorobotics. For instance, the superparamagnetic property is size-dependent and generally arises when the size of nanoparticles is as low as 10–20 nm. At such a small size, these nanoparticles do not exhibit multiple domains as found in large magnets and become a single magnetic domain acting as a "single super spin" that exhibits high magnetic susceptibility. Because of such a high magnetic susceptibility, when a magnetic field is applied, these SPIONs provide a stronger and more rapid magnetic response compared with bulk magnets with negligible remanence (residual magnetization) and coercivity (the field required to bring the magnetization level of the material to zero). The fact that these nanoparticles are superparamagnetic is critical for drug delivery and for nanorobotic agents. This is true not only because of the enhanced induced magnetic force that can be achieved but also because once the applied magnetic field is removed the nanoparticles retain no residual magnetism at the specific temperature level. The lack of residual magnetism leads to minimal danger of thrombosis or blockage of blood capillaries while minimizing possible complications related to potential post-interventional agglomerations of the nanoparticles.

One year later in 1991, a first microrobotic agent in the form of a swimming microorganism (Paramecium—length of 300 μm) was directionally controlled by exploiting its galvanic responses.[10] Although not being considered as a nanorobotic agent, such a demonstration proposed for the first time the use of a microorganism acting as a robotic agent. Then four years later in 1995, the single movable coil configuration implemented in 1990 was replaced by a configuration based on six static coils.[11] The additional coils enabled faster directional changes by modifying the ratio of electrical currents circulating in the coils. This implementation was a precursor to many static multicoil configurations that would later be developed for controlling the displacement of relatively large microrobotic agents. Indeed, for whole-body interventions, the relatively fast decay of the magnetic field when going further away from the magnetic source needed to be compensated by a larger magnetic volume of embedded magnetic material as found in larger microrobotic agents. By providing a relatively low field strength in the interventional space, these platforms failed to provide the capability to bring superparamagnetic-based (typically made of clusters of superparamagnetic nanoparticles) nanorobotic agents at saturation magnetization in order to maximize the induced directional force from a given directional magnetic gradient field within a volume sufficient to conduct whole-body operations.

Followed by further studies towards synthetic molecular motors,[12] it is five years later in 2000 that wireless capsule endoscopes capable of transmitting images of the gastrointestinal track (GI) began to attract attention.[13] Although the displacement of such capsules was not robotically controlled and the overall size of each capsule was far larger than what would be required for travelling in the vascular network, this was still considered a radical shift in the field. Indeed, for the first time, an untethered entity designed to travel in the human body (in this case through the GI track) was accepted for clinical uses. During the same year, a hybrid implementation consisting of a F_1-adenosine triphosphate synthase (F_1-ATPase) bio-molecular motor actuating a fabricated inorganic nano-propeller was tested successfully.[14] This hybrid propelling construct was another example among others that suggested potential contributions to the development of far future nanorobotic agents. But questions were raised concerning practical issues to be used in clinical interventions for such types of molecular constructs. These issues included but were not limited to the lack of a sufficient thrust force to navigate in the vascular network, power, reliability, and manufacturing cost.

It was followed one year later in 2001 by a swimming construct driven by a magnetic torque that showed the capability to penetrate tissues.[15] This relatively large system would later inspire torque-based actuated microrobotic agents such as the ones mimicking the artificial flagella of bacteria. Indeed, torque-based actuation requires less magnetic field strength to achieve a displacement of smaller robotic agents operating under low Reynolds hydrodynamic conditions compared to magnetic pulling approaches that are based on a directional force induced from one or more external magnetic sources.

Three years later in 2004, a more complete implementation in the form of a fragment of polydimethylsiloxane being moved in a non-controlled fashion by attached bacteria[16] was shown. It is also during the same year that the concept of chemical propulsion in catalytic motors[17] was demonstrated. This proof-of-concept triggered future efforts in chemically propelled microscopic agents. Then one year later in 2005, the first microscale artificial robotic agent mimicking microorganisms was reported.[18] It consisted of an artificial micro-swimmer with the controlled swimming motion of an artificial flexible flagellum made from a linear chain of colloidal magnetic particles linked by DNA and attached to a red blood cell. A molecular scale moveable construct dubbed the nanocar[19] made of fullerene molecules was also reported during the same year. As for other molecular constructs reported earlier, its practicability for clinical uses was questionable, at least in a nearer future.

Also in 2005, the first directionally controlled agent based on a microorganism carrying a load was reported.[20] It consisted of a flagellated algae (slightly larger than a bacterium) using phototaxis for directional control. The load consisted of a few micrometers in diameter polystyrene bead being attached to the cell using surface chemistry. The load was released using photochemistry. In parallel, efforts were done towards the use of a magnetic field (magnetotaxis) instead of light (phototaxis) in an attempt to make the directional control source applicable for operations performed deep inside the human body. These efforts based on magnetotaxis led to the first micro-object being attached and transported by a bacterium being directionally controlled along a predefined path that was reported only a few months later in 2006[21] from initial works that began in 2002 and filed in 2004.[22] This first directionally controlled bacteria-based nanorobotic agent consisted of a polymer microbead attached to a magnetotactic bacterium (MTB). For the first time, a directionally controllable self-propelled microorganism-based nanorobotic agent was small enough to travel through the tumoral physiological microenvironments using the shortest physiological routes with the added capability to penetrate deep regions in tumors. Since the controlled displacement was achieved by inducing a directional torque on a chain of nanoparticles (known as magnetosomes and located in the bacterial cell itself) from a weak magnetic field, directional control could theoretically be done anywhere and at any depth within the human body. Hence, MTB and, in particular, the MC-1 cell represented a highly potential agent to operate in the human body well beyond the range of magnetically pulled artificial/synthetic agents. Indeed, besides harnessing MTB cells to act as computer-controlled nanorobotic agents for diverse tasks that led to the first controlled coordinated micro-assembly task achieved with a swarm of microorganisms and which has been reported in 2010,[23] the

main motivation was also to complement a new technology initiated in 2002 and aimed at navigating nanorobotic agents using a clinical Magnetic Resonance Imaging (MRI) scanner, a method being referred to later as Magnetic Resonance Navigation (MRN). MRN was part of a project dubbed MR-Sub (Magnetic Resonance Submarine) making reference for the first time in the field of micro- and nanorobotics to the submarine navigating in the blood vessels in the 1966 movie "Fantastic Voyage."

THE PRE-TRANSLATIONAL ERA

In early November 2006 (published in March 2007), the first demonstration of an untethered object (a 1.5 mm in diameter ferromagnetic bead) being navigated in the blood vessel (carotid artery) of a living animal (a swine) was demonstrated.[26] Such experimental demonstration being part of the MR-Sub project and which used the principles of MRN triggered a new wave of excitement from the engineering fields that led towards a substantial increase in research activities in medical nanorobotics, especially towards medical applications. A bit earlier, in March 2006, 2- and 3-dimensional shapes at the nanoscale being created using DNA origami[24] were demonstrated. These experimental demonstrations were based on the idea of using DNA as construction material which was first introduced in the early 1980s by Seeman. Such a concept would later suggest that self-assembly could be used to create huge quantities of nanorobotic artificial structures in the future as a potential alternative to conventional manufacturing methods. The first proposed bacteria-propelled microelectronic agent[25] electrically powered using micro-photovoltaic cells was also proposed in 2006. Around the same period, bacteria-mediated delivery of nanoparticles and cargo into cells was also demonstrated.[27] Although the displacement of the bacteria was not controlled, it opened further possibilities for medical bacteria-based nanorobotic agents. During the same year, phototactic directional control of a relatively large structure propelled by attached flagellated bacteria was demonstrated[28] followed by the DC electric field directional control of the same bacteria-propelled structure in 2011.[29] These demonstrations extended further the list of possible directional control methods for microorganism-based nanorobotic agents.

In 2008, it was proposed for the first time that combining magnetotaxis with an available onboard sensory means of a nanorobotic agent and, in particular, aerotaxis in magneto-aerotactic bacteria, nanorobotic agents could enhance further the therapeutic effects in cancer therapy. This could be achieved by targeting non-systemically oxygen-depraved areas within solid tumors.[30] This led, several years later, to the development of a nanorobotic delivery method to maximize the therapeutic outcomes by targeting the tumor hypoxic zones through non-systemic physiological routes. Then at the end of 2008, the effect of the quantity and configuration of attached bacteria on bacterial propulsion of microbeads was studied for the first time.[31] Such study provided important insights for the development of future hybrid nanorobotic agents propelled by more than one bacterium.

One year later in 2009, directionally controlled artificial magnetically actuated microscopic agents mimicking the flagellum of bacteria also being often referred to as helical micro- and nano-swimmers were revealed. This event initiated a competition between controlled artificial structures and microorganisms being harnessed as nanorobotic agents. Two such main artificial implementations were released in the same year in the forms of microrobotic agents being referred to as Artificial Bacterial Flagella[32] (ABF) and a much smaller version in the form of magnetically controlled artificial nanostructured propellers.[33]

During the following year in 2010, 15 years after the first implemented multicoil platform based on six static electromagnetic coils, another statically positioned multicoil (eight static coils) experimental interventional platform was introduced. It was known as OctoMag[34] and was designed primarily to control intraocular microrobots (500 μm in length) for future delicate retinal procedures. During the same period, more advanced MRI-based navigation control algorithms following the PID (Proportional Integral Derivative)-based navigation control initially used for the navigation in the carotid artery of a pig in 2006 as part of the project MR-Sub were developed. This trend began with predictive control.[35] It is also during the same year that

chemically powered nanomotors or catalytic microjet bubble-propelled engines became serious competitors in term of velocity to flagellated bacteria acting as nanorobotic agents.[36] Then approximately one year later in 2011, navigable nanorobotic agents known as Therapeutic Magnetic Micro Carriers (TMMCs) and consisting of magnetic nanoparticles (MNPs) encapsulated with therapeutics (Doxorubicin) in a biodegradable polymer matrix [PLGA (poly(lactic-*co*-glycolic acid) in this particular case] of approximately 50 μm in diameter successfully delivered therapeutics (with controlled release) for the first time in targeted regions of living animals.[37] This first proof-of-concept preclinical study that resulted to successful *in vivo* targeting and delivery of drug-cargos to the right or left liver lobes of rabbits was achieved by prior navigation of the therapeutic nanorobotic agents in the hepatic artery using the MRN-based concept. Also in 2011, a MRN-compatible nanorobotic navigable agent capable of changing shape was synthesized.[38] It consisted of a hydrogel-based agent that reduced its overall dimension when exposed to an elevation of its internal temperature created through hyperthermia when exposed to a modulated magnetic field acting on the same embedded magnetic nanoparticles being used for propulsion and real-time tracking using a clinical MRI scanner.

Then in 2012, several chemotactic bacteria were used to displace a large liposome.[39] Although no directional control was performed and no drug-cargo was transported, it showed that a structure known to be widely used for carrying drug-molecules could be displaced using several attached microorganisms. Also, chemotactic steering of bacteria-propelled microbeads was reported at the end of the same year.[40] Then from 2013 to 2014, other vascular magnetic navigation methods for nanorobotic agents were proposed,[41] including the introduction in 2014 of a method dubbed Dipole Field Navigation (DFN).[42] DFN provided for the first time, both whole-body depth-independent high magnetic field strength sufficient to bring superparamagnetic nanoparticles at saturation magnetization and high directional gradients. Combining high field strength and gradients, much superior induced magnetic directional propelling/steering forces could be induced on nanorobotic agents. Also in 2013, a platform known as the Magnetotaxis system and related methods to achieve aggregations of magnetic nanorobotic agents (in this case being magnetotactic bacteria) in a three-dimensional volume was described and validated for the first time.[43] The magnetotaxis system enabled the displacement control and making possible the accumulation (aggregation) of magnetic nanorobotic agents in deeply located physiological targets such as tumors. During the same year, results of chemotaxis-based tumor targeting in mice models using bacteria-based microrobotic agents in the form of a bead propelled by several bacteria (also being referred to as bacteriobots) were published.[44] Because the systemic route was used, since no directional control was applied following injections and based on previous results done by other groups with similar bacteria but not attached to a bead, it was anticipated that only a small fraction would likely come sufficiently close to the tumor to allow the detection of chemical gradients if applied in humans. This would most likely yield a much lower therapeutic index compared to a nanorobotic agents that could be navigated in the vascular network. Indeed, among bacterial strains that were evaluated as cancer therapeutics, *Salmonella* Typhimurium was and still one of the most promising bacteria to be used for bacteriotherapy in cancer but first-in-man studies (phase 1 clinical trials) conducted in 2012 showed that although excellent tumor colonization was observed in murine models, it was not confirmed in humans. This result suggested that not being able to avoid systemic deliveries remained one of the major obstacles to allow further improvement. Again, enhancing the therapeutic index could most likely be achieved using nanorobotic-based non-systemic deliveries also being referred to as direct targeting.[41] Furthermore, the therapeutic effects in humans would most likely be reduced further by the fact that the overall construct was too large to penetrate deep in tumoral tissues, while the lack of an appropriate onboard oxygen gradient detector would further prevent such agents to target the hypoxic areas that would most likely result towards an optimal treatment efficacy. These functionalities and characteristics not present in such agents were known to exist in MTB-based agents. Nonetheless, such experiments were very interesting, since they validated the possibility of using chemical gradients for a relatively large bead or other potential structures being propelled by several bacteria in order to improve the targeting efficacy of tumors by chemotactic nanorobotic agents.

Also in 2013, it was found that besides the lack of biocompatible fuels, the motion of catalytic microjets would only be possible in highly diluted dispersions of the red blood cells and serum.[45] These findings would impose serious constraints for medical applications (at least in the short term). Despite these constraints, research in catalytic-based agents continued at a great pace. For instance, in 2014, the first wireless magnetic-based closed-loop displacement control of catalytic microjets[46] and multimodal chemo-magnetic directional control of chemically propelled agents[47] were reported. Soon, these demonstrations were followed in 2015 by the first *in vivo* demonstration of a chemically propelled agent which was performed in the stomach of a mouse.[48] Also in 2014, a magnetically controlled sperm-based hybrid agent dubbed magnetosperm was proposed.[49] This agent replacing flagellated bacteria for propulsion was interesting, since it proposed the use of an artificial magnetic construct to make non-magnetotactic biological propelling systems directionally controllable with a magnetic torque. This demonstration also suggested for the first time the use of microorganism-based robotic agents to help egg fertilizations.

In the same year, the first directionally controllable self-propelled nanorobotic therapeutic agent was also introduced.[50] It consisted of drug-loaded nanoliposomes covalently attached to the surface of MC-1 magnetotactic bacteria (MC-1-LP) acting as directionally controllable therapeutic vectors. A non-self-propelled artificial version was also proposed the same year and consisted of artificial bacteria flagella functionalized with temperature-sensitive liposomes (f-ABF) for drug release.[51] These developments confirmed one more time the mutual investigation of the two main strategic trends, namely, artificial/synthetic versus microorganism-based nanorobotic vectors in the development of nanorobotic agents for drug delivery. Drug delivery into a single cell *in vitro* using f-ABF was also demonstrated during the same year.[52] It provided an artificial version of the bacteria-mediated delivery into cells that was reported seven years earlier in 2007. Also in 2014, another directionally controllable magnetic construct in the form of a magnetic microbead sufficiently large to be propelled by several flagellated chemotactic bacteria was demonstrated.[53] It replaced the sperm-based hybrid agent proposed the same year by a smaller magnetically controlled (made possible by the microstructure or microbead) bacteria-based version. But although smaller, the addition of a magnetically steerable construct combined with several bacteria would result in overall dimensions that would likely be too large to enter tumors due to physiological volumetric constraints. As such, other types of medical interventions would need to be considered. During the same year and eight years after the creations of nanoscale shapes using DNA origami, it was showed that DNA origami could also be used to fabricate nanoscale robots that are capable to dynamically interact with each other.[54]

Also in 2014, in the field of magnetic actuation, preliminary experimental results of a new magnetic navigation method dubbed Fringe Field Navigation (FFN)[55] initially developed for guidewire and catheter navigation but applicable in some particular cases to nanorobotic agents was released. FFN provided magnetic gradients much superior to any pre-existing platforms while offering a field strength superior to all pre-existing methods except MRN and DFN. It offered a solution to enable whole-body interventions for the single superconductive coil configuration proposed in 1990. Indeed, such a solution relied on robotically moving the patient within the external fringe field created by a large superconductive magnet (such as the one integrated in a clinical MRI scanner and used to generate the high uniform field known as the B_0 field) instead of moving such bulky superconductive magnet which proved to be impractical for whole-body interventions. But as the single superconductive coil configuration reported in 1990, FFN shared the same relatively slow directional changes, since modulating the electrical current in a superconductive coil fast enough to support many nanorobotic-based interventions was and still not possible or practically suitable. This fact forced both approaches to rely on a mechanical instead of an electrical approach to achieve directional changes, which imposed constraints on the types of vascular networks that could practically be navigated prior to reach the targeted physiological region. Also during the same year, a first study providing guidelines through dynamics molecular simulations for the design of nanorobotic agents to cross the brain-blood barrier by combining magnetic gradients and chemical functionalization was published.[56]

More recently in 2015, an experimental demonstration[57] suggested that small scale robots could be self-assembled to increase not only the overall force as demonstrated by the collective force of a swarm of magnetotactic bacteria acting as nanorobots capable of moving relatively large bricks as mentioned earlier and which was initially reported in 2010 but also the total impact force (i.e., a cumulative force being delivered instantaneously) as well for applications such as creating punctures through tissues. Although this approach was validated at a larger scale, being able to scale such capability for microscale agents beyond what would be possible with a single robot remains a challenging task. Also in 2015, five years after the first controlled displacement in 2010 of a swarm of microorganisms in the form of MTB, the *in vivo* controlled swimming of a swarm of artificial agents in the form of ABF was demonstrated,[58] confirming one more time the competition between microorganism-based and artificial agents. Finally in the same year, it was also shown in rat models that the same nanoparticles used to induce a displacement force on nanorobotic agents while providing a means to detect their position using a clinical MRI scanner could also be used to temporary and reversibly open the blood-brain barrier.[59] By embedding this new functionality made possible by exploiting nanotechnology, it allowed precise accesses (at the exact same locations of the navigable agents) to the last physiological regions in the human body that was so far out-of-reach to nanorobotic agents.

NEAR FUTURE PROSPECTS: THE TRANSLATIONAL ERA

Although it is hard to predict accurately the future discoveries and developments in the field of medical nanorobotics, specific trends can be identified based on recent developments and the present state of the field that was summarized in the "The Proofs-of-Concepts Era" and "The Pre-translational Era" sections. But by just speculating and mentioning in this section, some of the many future possibilities may also influence to some extents the outcomes and the time at which these new developments may occur. Nonetheless, such future developments must still follow specific technological and scientific trends, translational initiatives towards clinical applications, as well as practical and economic issues, and all these factors were considered in these predictions.

First of all, many research groups will continue to develop principles and methods associated with medical nanorobotics. Many molecular constructs such as synthetic molecular motors and other bio-actuators such as nanorobotic agents propelled by heart muscle actuators are just some examples that performed well under laboratory conditions, but will most likely require a longer development time prior to be considered for clinical uses. These proposed constructs might not be practically viable for awhile due in part to the complexity in the assembly processes and because of reliability issues, to name but only two issues that will most likely require much longer time to get to a sufficiently high maturity level before being potentially considered for clinical uses.

The future developments that will yield the higher impacts in the shorter terms and hence stimulate the translations of these technologies to the clinics are the ones that will show superior advantages and results compared to the clinical tools and methods that presently do not rely on medical nanorobotics. To accomplish this, research groups involved in medical nanorobotics will have to tackle and integrate many disciplines. The number of highly interdisciplinary research laboratories involved in medical nanorobotics has increased in recent years, and the trend will most likely continue. This in turn will lead to more advanced, sophisticated, and mature interventional platforms and nanorobotic agents capable of competing with actual medical practices and pharmaceutical agents.

But signs already show that it will be extremely hard for medical nanorobotics to be accepted in any medical interventions conducted on a regular basis. The main reason is that the field still appears as a far reaching set of technologies and a radical shift involving high implementation risks. To accelerate its integration in medical practices, medical nanorobotics will have to show results that are not only equivalent or marginally superior to actual interventional methods, but the results achieved would have to be so high and so impressive that the medical experts and practitioners will have no choice but to recognize the superiority of this new

technological trend. This will be essential in order to motivate them to take the risks associated in adopting such a shift that will not only influence their actual medical practices but also offer substantial positive impacts on a potentially large number of patients. When this happens, then the pharmaceutical industry that so far showed resistance or ignored the possibility to synthesize new potential therapeutic vectors made possible by medical nanorobotics will definitively notice such a motivation to changes from the medical community and will most likely respond accordingly when the benefits of doing so will be evident, i.e., when the interest from the medical community for such agents will be relatively substantial. But this level of acceptance will most likely be reached after a relatively long period of time, and researchers in medical nanorobotics would have to be patient.

Besides other potentially viable applications such as reproductive medicine where efforts in the coming years are most likely to be pursued, one particular medical application where medical nanorobotics will most likely attract the attention and initiate such translational research initiative in the shorter term is in cancer therapy. This is because for cancer therapy, medical nanorobotics can yield a high impact by offering the potential to significantly enhance the therapeutic index for the large majority of cancers. Such enhancement in the therapeutic index would translate to a substantial increase of the therapeutic efficacy well beyond the synthesis of new drug molecules alone by reaching more effectively the regions to be treated while avoiding or at least minimizing systemic circulation that is responsible for the increase in toxicity for the patient. Decreasing secondary effects by avoiding or at least reducing systemic toxicity that affects healthy tissues and organs will most likely contribute to a substantial reduction of the length of hospital stays for the patients while shortening the time of treatment through a significant improvement of the therapeutic index. These factors combining economic motivation due to a reduced cost of treatments, and the superior treatment efficacy will contribute to facilitate its adoption in the clinics.

The medical nanorobotics community has noticed that cancer therapy is indeed a good starting point when one looks at the research efforts that have been done in the field over the last few years. Based on the present maturity level of medical nanorobotics, it is anticipated that the first *in vivo* non-systemic delivery of drug molecules into a tumor will soon be demonstrated in animal models. This will most probably be done with microorganism-based agents. Then soon after, contrast agents could be delivered most probably by the same nanorobotic vectors within specific tumoral regions in animal models, hence expanding the potential roles of medical nanorobotic vectors as imaging, diagnostic, and theranostic agents. The far superior therapeutic effects resulting from the high therapeutic index achieved by such microorganism-based therapeutic nanorobotic agents could also be demonstrated earlier than we thought initially. While such non-systemic deliveries of therapeutics to tumors could become a turning point in medical nanorobotics, it is the latter demonstrating the far superior therapeutic effects compared to present systemic delivery methods that will most likely trigger a higher level of excitement from the medical community. In turn, such excitement would certainly facilitate collaborative efforts to bring medical nanorobotics to the clinics. Although substantial translational research efforts based on nanorobotic vectors have already began in 2015 using MRN for liver chemoembolization in pigs, it is anticipated that translational research activities will increase substantially for other types of cancer as early as the beginning of 2017 when the first medical nanorobotic interventional facility dedicated to cancer therapy and put in place in 2016 will be at a sufficient operational level. This will accentuate efforts to demonstrate the advantages of nanorobotic agents in many cancer therapy modalities including but not limited to targeted radiotherapy, hyperthermia, thermal ablation, gene therapy, immunotherapy, and the delivery of stem cells, to name but just a few examples. Efforts to target the brain with both synthetic and bacteria-based nanorobotic vectors based on techniques and methods already available to open the blood-brain barrier mostly using localized hyperthermia from the same non-systemic navigable agents, or from more mature technologies such as High Intensity Focus Ultrasounds (HIFU), will soon be initiated as well.

In parallel, such targeting results in tumors and the corresponding therapeutic outcomes achieved with microorganism-based nanorobotic agents will motivate efforts to duplicate these

results with other species of bacteria including chemotactic bacteria and other species of MTB, as well as with artificial nanorobotic agents. Such artificial agents will most likely take the form of ABF and other biomimetic constructs, as well as catalytic-based agents. A percentage of these agents if miniaturized further while being magnetically guided may succeed at delivering therapeutics to tumors. For chemically powered nanorobotic agents, this will motivate an increase in research for the development of a biocompatible fuel. The use of such artificial nanorobotic agents may yield noticeable therapeutic effects at some point in time but they will most likely not reach, at least initially, the same or superior therapeutic index achieved with microorganism-based agents and in particular, MTB-based agents for some types of tumors. This is due in part to the expected inefficiency for artificial and synthetic agents to find within the many physiological obstacles, pathways towards the tumoral regions. This is true when considering the inadequate spatial resolutions of existing medical imaging modalities to provide feedback information to perform closed-loop directionally control of such agents. Similarly, the lack of an appropriate embedded sensory capability to target regions leading to optimal therapeutic outcomes such as the hypoxic areas within solid tumors will make it difficult for artificial agents to compete against microorganisms such as MC-1 MTB that have the appropriate sensory-based displacement behaviors to autonomously target the hypoxic areas. As such, knowing that sensory-based agents are likely to be superior for many medical applications, substantial efforts will likely be put forward in the following years to integrate taxis-based displacements in artificial nanorobotic agents. Such efforts could consider pH-level or other sensory capabilities being integrated to magnetically guided artificial agents. Chemotaxis could also be particularly attractive due to the fact that in 2014, non-magnetically guided chemotaxis-based agents have already shown to be feasible with chemotactic bacteria. While this may offer the potential to enhance tumor targeting, it may also prove to be extremely difficult to implement in an artificial agent. This will most likely motivate further studies exploiting the sensory capabilities of magnetically guided microorganism-based constructs.

Since the implementation of sensory-based artificial agents sufficiently small to reach deep inside tumors may prove to be a difficult task, image-guided approaches based on the gathering of specific regions such as the hypoxic areas by an external imaging modality would most likely be investigated as well to control the displacement of these artificial nanorobotic agents. But such an approach will be constrained at least in the shorter term by the limitations of actual clinical imaging methods to precisely locate these regions. As such, in order to compete with bacteria-based agents, it is anticipated that new types of interventions better suited for such artificial nanorobotic agents may be investigated as well during the coming years. Despite the previous facts, it is also anticipated that other avenues would be investigated to compensate for the lack of functionality embedded in artificial agents. One particular avenue that will most likely be investigated in the shorter term is the use of extremely small helical swimmers capable of displacements through tissues and directly towards the targeted region. This would offer a potential alternative instead of navigating through existing physiological routes in order to compensate for the lack of navigation capability on such narrowed physiological routes. By doing so, flooding the tumoral volume with a much larger number of drug-loaded agents would be necessary to compensate for the inability to detect the hypoxic regions. This will ensure that the hypoxic regions are targeted as well. Such an approach will also require the achievement of a sufficiently high targeting ratio, since a proportionally higher number of agents may contribute to increase systemic toxicity compared to the use of a smaller number of agents capable of selectively target the hypoxic areas. Furthermore, such small agents would have to be faster by achieving a much higher number of body-lengths per second in order to maintain the overall intervention time within acceptable limits. Although the latter may prove to be very difficult to achieve, research efforts involving such agents are likely to be pursued in the coming years. Another aspect that could limit the targeting efficacy of these agents will be the potential influence of the blood flow while crossing specific physiological regions such as capillary vessels. Indeed, the lower propulsive force of such smaller agents with regards to blood flow rates may not prevent them to deviate from their original course. In turn, this may result to a significant decrease of the therapeutic index and an increase of the systemic toxicity.

But another particular and important factor that will challenge the adoption of artificial nanorobotic agents for cancer therapy in the coming years will be concerned with the manufacturing aspect. Indeed, because of the limit in the quantity of therapeutics that each of these small nanorobotic agents can carry; a very large quantity in the order of at least tens of millions of such nanorobotic agents would most likely need to be injected at a time. This issue becomes more critical when the treatment must rely on a series of repeated scheduled injections required to deliver sufficient drug molecules to yield significant therapeutic effects. Although such a quantity can be practically and economically reached using microorganisms such as bacteria, a practically and economically viable manufacturing process for artificial nanorobotic agents may be available only after some more additional years of research and development. As such, self-assembly methods including DNA origami may be considered but the suitability of these types of approaches for clinical uses in term of design flexibility, reliability, etc., will most likely require a substantial additional number of years. Some shortcuts would probably be considered, such as coating existing natural micro- nanostructures with magnetic material, for instance, including but not limited to superparamagnetic nanoparticles, providing hybrid approaches but again, with limited embedded functionalities compared to microorganism-based nanorobotic agents.

But such a manufacturing issue will not have a great impact initially for research laboratories. But as artificial agents will be seriously considered as an alternative to microorganism-based nanorobotic agents in demonstrating their levels of efficacy in cancer treatments, this manufacturing aspect is likely to become a more serious issue. By taking advantage of the non-systemic delivery capability made possible by nanorobotic agents while waiting for the manufacturing technology to evolve to a sufficient level, attempts will mostly be made to consider more effective and therefore more toxic drug molecules. This will be motivated by assuming that a smaller but more effective and therefore toxic quantity of therapeutics would require a smaller quantity of nanorobotic agents. But such a strategy would not make artificial agents much more attractive than microorganism-based agents, since the same approach could and will likely be considered for both natural and synthetic/artificial agents. But one actual particular advantage of artificial agents over microorganism-based nanorobotic agents at least in the short term is the fact that instead of carrying the therapeutic payloads on the surface of the agents, such payload could be integrated within the structure itself and released through mechanisms such as biodegradation following a predefined release profile. This could potentially yield a higher density of drug molecules being transported for the same quantity of agents. This means that logically, research and development towards biocompatible biodegradable polymer-based (e.g., PLGA, PLA (Polylactic acid), etc.) artificial agents including artificial flagella-based agents will most likely be pursued in the coming years.

But despite all the advantages over artificial agents of microorganism-based nanorobotic agents and, in particular, MTB-based agents that include self-reproducibility, self-propulsion, magnetotactic navigation, and sensory-based targeting, to name only the main advantages, such natural nanorobotic agents will also face in the coming years important issues that may delay their use in clinics. The most significant issue is related to regulatory approvals. Regulatory approvals are likely to be easier for synthetic or artificial agents developed in the coming years. This is particularly true if they use materials such as already approved biodegradable polymers and superparamagnetic iron-oxide nanoparticles with already approved drug molecules. Nonetheless, a significant increase in the development of microorganism-based nanorobotic vectors is still expected in the coming years once the first microorganism-based nanorobotic agent will be approved for clinical uses. Indeed, based on preliminary results obtained with MC-1 MTB and with further tests to be conducted in the near future, this approval could potentially occur within the next few years. When this occurs, it will most likely trigger more efforts in the following years towards microorganism-based agents compared to artificial or synthetic nanorobotic agents. Besides considering various therapeutic, diagnostic, and imaging payloads, including theranostic and the implementation of microorganism-based agents enabling multimodality treatments, further genetic studies and modifications of existing microorganisms through gene manipulations will most likely be pursued to offer a larger library of microorganism-based nanorobotic agents. In particular, efforts to genetically implement chain of magnetosomes in other microorganisms offering advantages for specific treatments are likely to be

initiated and pursued first within the next few years. This initiative would significantly extend the library of microorganism-based nanorobotic agents compatible with new nanorobotics interventional systems such as the magnetotaxis platform.

In the meantime, although research in the development of larger structures being displaced by several microorganisms such as bacteria will be pursued in the following years, these constructs would most likely have minor impacts in the medical field, at least in the shorter term. The main reason is the overall size of this type of vectors that will remain too large to target physiological regions (such as within tumors) being well beyond what can be reached by magnetic-based synthetic nanorobotic agents such as the ones propelled using MRN or DFN, for instance. Furthermore, considering the fact that not sufficient propelling forces will be achieved to navigate effectively in larger blood vessels such as arteries will limit the ranges of physiological regions where they can operate. This in turn will limit the number of potential medical interventions that they could support. As such, it is anticipated that such a type of vectors including the use of larger microorganisms being linked with a magnetic construct directionally controlled using a magnetic torque, although presenting interesting and valuable engineering concepts, will become less attractive for specific medical applications such as tumor targeting within the next few years. On the other hand, they may become more attractive for other medical applications. Other medical applications where such a type of vectors could compete in term of cost or feasibility, for instance, and where such larger microorganism-based constructs may have potentials in the medical field would be to complement technologies such as MRN, DFN, etc., to reach deeper physiological regions in the vascular networks such as in the arterioles, as schematically represented in Fig. 2.

For instance, besides reproductive medicine and other potential applications, such larger constructs could be investigated to perform chemoembolization in deeper physiological regions or to release microorganism-based agents or magnetic artificial microswimmers closer to the microvasculature. The latter application especially in synthetic forms at first is most likely to attract substantial research and development efforts in the coming years. This is especially true when one considers that the travel distance and the blood flow in larger blood vessels will be important obstacles that will prevent smaller artificial/synthetic and microorganism-based

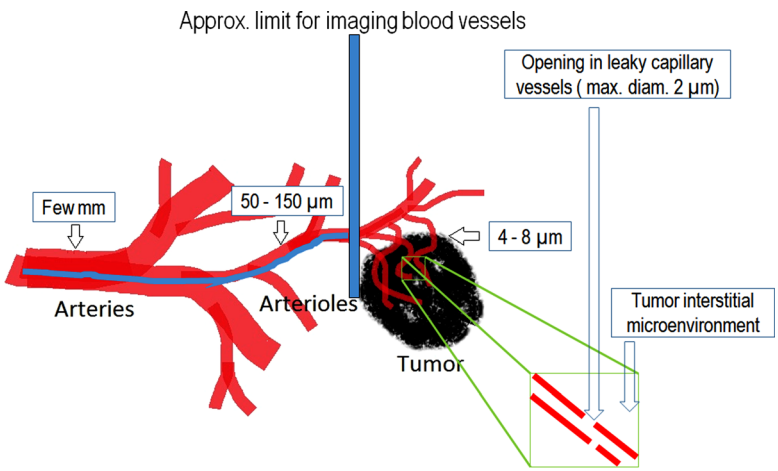


FIG. 2. Simplified representation of the vascular network that must be travelled to reach a tumor—Larger artificial or synthetic agents under closed-loop navigation control from feedback data gathered from a medical imaging modality are likely to dominate in larger blood vessels (left of the approximately limit of imaging blood vessels depicted in the figure). Gathering feedback information to navigate nanorobotic agents beyond the spatial resolution of existing medical imaging modalities will most likely require more autonomous nanorobotics agents. Although artificial agents designed to move more effectively in low Reynolds number conditions will challenge microorganism-based agents in the microvasculature and the interstitial spaces, the capability of some microorganisms to sense and find their ways in such complex networks under the influence of an external stimulus or from an appropriate embedded sensory capability will mostly yield superior targeting ratios, at least in the shorter term. To deliver payloads deep in the tumor, physiological routes such as the tumor interstitial microenvironment would need to be accessed mostly by transiting through the holes (having a maximum diameter of 2 μm) in the leaky vessels of the angiogenesis network that brings nutrients and oxygen to the tumor.

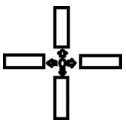
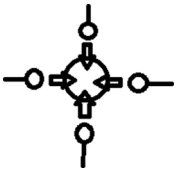
nanorobotic agents to reach regions such as solid tumors while achieving a sufficiently high therapeutic index. Unless, peritumoral injections are possible, which are only the case for some types of tumors, such larger microrobotic agents capable of transporting smaller nanorobotic agents through larger vessels towards the microvasculature will be mandatory and pursued for many other physiological regions being targeted. The use of larger magnetically steered structures being propelled by microorganisms in larger vascular networks such as the arteries and the arterioles will be challenged in the coming years by other technologically mature approaches such as EMA (electromagnetic actuation), DFN, FFN, and MRN. These approaches would be supported by compatible micro-carriers capable of transporting and releasing nanorobotic agents that are more efficient in the microvasculature and the tumor microenvironments.

But these technologies enabling navigation in larger blood vessels will also be challenged by new platforms and, in particular, the one enabling a new imaging modality known as Magnetic Particle Imaging (MPI). The initial concept of MPI was proposed in 2001 by researchers at the Royal Philips Research Lab in Hamburg with their first MPI scanner set up being tested in 2005[60] prior to the first recording of images of the blood flow and organs in mice. More recently, MPI has been considered for the controlled displacement of magnetic agents, since the predicted maximum magnetic gradients of 2 T/m for whole-body versions put MPI as a potentially serious competitor to conduct vascular navigation of artificial or synthetic nanorobotic agents. The estimated MPI-based maximum gradient amplitudes are similar to the ones possible with FFN. But MPI has the gained advantage of fast directional changes by moving what is known as the Field Free Point (FFP) (area of lowest magnetic field strength) by modifying the electrical currents circulating in the surrounding coils. MPI is also suitable for the fast tracking of magnetic agents. But MPI has some drawbacks. The variation of the magnetization during the displacement of the agent with MPI makes more difficult to achieve effective navigation in the vascular network. The field strength can be sufficient to bring several agents at saturation magnetization if sufficiently far from the FFP, i.e., closer to the coils, but still insufficient for the highest performance superparamagnetic agents which can be achieved with the use of MRN and DFN and possibly FFN is some particular cases. To visualize physiological tissues including targets such as tumors would require that the patient be moved to another platform such as an MRI scanner. This will require additional processes such as image registration that can add to uncertainties. The fact that MPI is a relatively new technology that is not widely available in clinics would most likely slow the introduction of MPI-based actuation for medical interventions, at least during the next few years. But the technology may prove to be suitable for some types of relatively large magnetic agents operating in larger vascular networks especially when one considers the facts that magnetic tracers injected in blood vessels would provide image of the pathways to be navigated, while MPI-based real-time tracking would ease the implementations of closed-loop vascular navigation control algorithms. But MPI-based actuation as EMA, MRN, and FFN requires that only one bolus of nanorobotic agents be injected and navigated at a time. This fact leading to many repeated injections and navigation phases would most often result to a substantial increase of the total time to conduct the intervention. Since with DFN, one single injection of all agents is possible, the interventional time can potentially be significantly reduced. This in turn would translate to a huge advantage in term of cost, hence potentially facilitating its adoption in the clinics. But many other factors must be taken into account and since each approach has their own advantages and limitations, it is likely that several of them or variations of these platforms being listed in Table I will at some point in the future be investigated or at least be considered for clinical uses. As for future medical micro- and nanorobotic agents, they are likely to take various forms but most of them will most likely take their inspiration from one of the existing agents being listed in Table II.

FAR FUTURE PROSPECTS: THE PREEMPTIVE ERA

Unlike previous nanorobotic agents designed for targeting a predefined physiological region by navigating through the shortest physiological routes, the nanorobotic agents of the preemptive era are expected to be designed to circulate in the whole systemic blood network. Indeed,

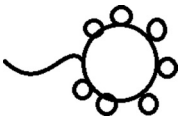
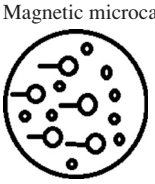
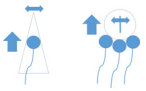
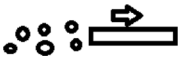
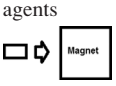
TABLE I. List of main navigation platforms for nanorobotic agents.

Technological platforms	Principle	Specifications	Physiological areas
MRN 	Exploits of the magnetic fields inside the tunnel of a clinical MRI scanner to navigate nanorobotic agents, the uniform B_0 field saturates the magnetic agents, while the imaging gradients induce 3D displacement forces on the nanorobotic agents	Highest magnetic field strength (1.5 T to 3 T) to saturate all types of magnetic agents, fast but relatively low directional gradients (0.04 T/m), provides real-time tracking of magnetic agents and physiological tissue imaging	Arteries, arterioles
DFN 	Distorts the uniform magnetic field inside the tunnel of a clinical MRI scanner to create high directional gradients	Highest magnetic field strength (1.5 T to 3 T) to saturate all types of magnetic agents, high (0.3 T/m) preset directional gradients	Arteries, arterioles
FFN 	Mechanically moves the patient typically in the fringe field surrounding a clinical MRI scanner	High magnetic field strengths possible (0.5–1.5 T) to increase the magnetization level of agents, slow directional but very high (above 2 T/m possible) gradients	Arteries, arterioles with no directional change preferred
EMA 	Electromagnetic directional fields generated by ratios of electrical currents passing in surrounding coils	Very low magnetic field strengths (approximately 0.1 T) requiring larger magnetic volume per agent, fast and high (0.3 T/m) directional gradients	Arteries and larger physiological areas
MPI-based actuation 	Displacement of the FFP (region of low field strength) by changing the ratios of electrical currents passing in surrounding coils	Relatively low field strength depending on the distance from the surrounding coils, fast and very high (max. 2 T/m) directional gradients, provides real-time tracking of magnetic agents	Arteries, arterioles
Magnetotaxis 	Generation of a 3D zone (known as the aggregation zone) with directional fields generated by surrounding coils capable of directing and constraining the displacement of self-propelled magnetically guided (torque-based) agents in such a targeted zone	Typically above approximately 15 gauss outside the aggregation zone and towards zero inside the aggregation zone	Microvascular networks, interstitial and tumoral microenvironments, capillary vessels

the prevention of the growth of diseases relies first on early detection. As such, without any previous information about the location of the target, non-systemic delivery (direct targeting) is no more a suitable option. This fact calls for nanorobotic agents that will exploit the blood flow for travelling in order to monitor all parts of the body.

As such, although the next-generation nanorobotic agents will differ from the previous non-systemic-based agents, they will still retain three basic fundamental specifications typically integrated in robotic agents, namely, sensing, computation, and actuation. The best approach to implement these sense-compute-actuate molecular units would most likely be through DNA nanotechnology. Indeed, several breakthroughs reported in recent years suggest that, indeed, DNA nanotechnology will allow huge number of such nanorobotic agents circulating in the vascular network to create what could be described as an artificial immune system. DNA nanostructures offer control over shape, size, mechanical flexibility, and anisotropic surface modification. Such

TABLE II. Main types of potential medical nanorobotic agents.

Medical nanorobotic agents	Description	Compatible platforms and specifications	Physiological areas
TMMCs 	Biodegrade polymeric microstructures containing magnetic nanoparticles and therapeutic payloads	MRN, DFN, FFN, MPI—can be scaled down to approximately 50 μm in diam. (typ. around 150 μm for human interventions such as liver chemoembolization)	Arteries, arterioles
MTB-based agents 	Magnetotactic bacteria cells transporting payloads and being directed to the target site using magnetotaxis and aerotaxis	Magnetotaxis platform—1 to 2 μm in diameter allowing the cells to target tumoral regions, typ. 100 to150 body lengths per second in good environmental conditions, MTB-LP carries approximately 70 drug-loaded 170 nm liposomes, chain of MNPs in each cell allows magnetic directional torque to be used, while an oxygen sensor combined with the microaerophilic behavior of the MC-1 MTB allows the delivery of payloads in hypoxic regions of tumors	Microvascular networks, interstitial and tumoral microenvironments, capillary vessels
Magnetic microcarriers 	Special TMMCs capable of transporting typically MTB-based agents towards microvascular networks where MTBs are more effective	MRN, DFN, FFN, MPI initially followed by the magnetotaxis platform—Same dimensions as TMMCs with embedded superparamagnetic nanoparticles for the induction of a directional force and MRI-tracking, contains drug-loaded MTB-based agents instead of drug molecules alone to be released at a specific embolization site	All vascular network
Magnetically steerable microorganism-propelled microstructures 	Magnetic microstructure allowing a directional magnetic torque to be induced and being propelled by one or more microorganisms	Magnetotaxis platform—self-propelled agent larger than MTB-based agent (typ. tens of micrometers in diameter)	Typ. too large for capillary networks, interstitial spaces and tumoral microenvironments, no sufficient force for arteries
Catalytic microjets 	Chemically propelled agent capable of being directed through the induction of a magnetic torque	Magnetotaxis platform—present overall lengths in tens of micrometers, self-propelled agents that can be directed using a magnetic torque	Smaller arterioles, too large for capillaries, and not adequate for arteries
Magnetic microrobotic agents 	Capable of high degrees of freedom but much larger than nanorobotic agents	EMA—agents rely on a larger magnetic induction volume in the order of a few tens of micrometers	Arteries and physiological areas allowing larger microrobotic agents
Helical micro- and nano-swimmers 	Biomimetic constructs typically in the form of artificial flagella actuated by a rotating magnetic field that induces a torque for directional displacement	Magnetic platforms capable of generating weak rotational magnetic field—a relatively weak rotating magnetic field is needed to achieve displacements of only a few body lengths per second	Smaller arterioles, not adequate for arteries and tumoral environments

level of control offers the possibility to increase systemic circulation time by order of magnitudes, which is critical for long term monitoring. Advances in DNA computing may produce DNA-based nanorobots with the capability to detect a multitude of cancer cells and diseases and be programmed to control and modulate the release of specific therapeutic agents. Since the surfaces of such DNA-nanorobots could be fully addressable, multiple ligands, labels for bioimaging, antibodies, hormones and so forth could also be incorporated for efficient site-specific drug delivery and release. Output state changes as encountered in many robotic systems could also be envisioned by applying general principles of DNA molecular circuitry. This in turn could be used to compute physiological medium inputs to select the right output state for drug releases in a series of cascade reactions.

Although this is likely to be a very long term vision, it is not unrealistic considering recent advances in DNA nanotechnology. Indeed, nucleic acid (including DNA and RNA) nanotechnology has bloomed in the last decade. Although it is difficult to mention all breakthroughs in the field of DNA-nanotechnology, some are listed here to show the evolution of building blocks that could one day allow the implementations of such DNA-based nanorobots.

DNA rendering of polyhedral meshes at the nanoscale[62] and the realization of complex wireframe DNA origami nanostructures,[63] or RNA nanostructures,[64] are three recent examples of structural DNA or RNA nanotechnology. Recent exciting works directly related to such nanorobotic agents are also worth mentioning. One paper[65] reports a logic-gated nanorobot for targeted transport of molecular payloads, and the second paper[54] is about universal computing of DNA origami robots in a living animal.

Other examples that could inspire the synthesis of nanorobots containing therapeutic cargos include but are not limited to the realization of DNA origami molecular containers[66] and the construction of box-shaped 3D-DNA origami.[67]

CONCLUSIONS

While several versions of artificial nanorobotic agents designed to operate in low Reynolds hydrodynamic conditions rely on a biomimetic approach inspired by the locomotion of microorganisms and, in particular, the flagella of bacteria, other initiatives put instead most efforts at developing platforms and methods to harness specific microorganisms such as flagellated bacteria to act as sophisticated nanorobotic agents. Some would argue that flagellated bacteria are not nanorobotic agents. But following the studies of Berg[61] published in 1973 that showed that flagellated bacteria have rotary molecular motors with a design very similar to modern engineered motors but at a much smaller scale, and the fact that all the basic robotic components such as actuation, some level of intelligence, and sensory capabilities are all embedded in such a small entity with the possibility to influence their motion and behaviors from an external computer, for instance, may suggest that indeed, they are nanorobotic agents made of materials not typically used in robotics. Going further, if engineers would have more advanced technologies available to design much higher performance nanorobotic agents, the latter could end-up to be very similar to what nature already provides following a long evolutionary design process.

Recognizing or not the fact that microorganisms can be parts of the library of components available in the field of robotics such as polymeric and metallic components, for instance, the fact remains that so far, it appears that in the following years, microorganism-based nanorobotic agents will dominate in the physiological microenvironments, while synthetic or artificial agents will prove to be essential to conduct operations in larger blood vessels and physiological environments. Although the two approaches will be complementary for many types of interventions, efforts will continue to replace microorganism-based agents with artificial implementations. This competition will continue for many years to come as evolving technological trends will offer new possibilities for artificial agents, while genetics will expand the range of possibilities for microorganism-based agents. Hybrid implementations taking advantages of technological advances and genetics will also play a greater role in offering new possibilities.

But these developments will ultimately lead the way to applications where medical nanorobotics will take a more preemptive role where nanorobots may one day circulate in the human

body to prevent at the earliest possible time the grow of potential diseases. But such a vision that will take place during what is being referred to here as the preemptive medical nanorobotics era is likely to need much more efforts and creative interdisciplinary approaches that will require many more years of research and development. But one thing is sure, the research efforts that have been and still being conducted for both artificial/synthetic and microorganism-based nanorobotic agents will help achieving this ultimate goal that will take place in this vast 3D biomicrofluidic environment that is the human vascular network.

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